2018

Circadian clock proteins CRYs are involved in control of diet dependent Acot expression

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CIRCADIAN CLOCK PROTEINS CRYs ARE INVOLVED IN CONTROL OF DIET-DEPENDENT ACOT EXPRESSION.

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ABSTRACT

Circadian clocks are evolutionarily conserved molecular timekeeping systems that generate rhythms in physiology and behavior in almost all living organisms and synchronize them with external environment. Living organisms have multiple circadian clocks which control numerous physiological functions. The light entrained circadian clock involves a transcriptional-translational feedback loop which regulates locomotor activity and metabolic processes and coordinates them with daily rhythms. The food entrainable oscillator (FEO) clock also generates near 24 hour circadian rhythmicity by driving food anticipatory behavior in mice. Mice entrained on 12:12hr light-dark cycle have been shown in previous studies to generate circadian rhythms in food anticipatory behavior, suggesting that this clock runs not on light independent, but food dependent cues. Availability of nutrients regulates metabolic pathways, which promotes cell growth and proliferation. Acyl-CoA Thioesterases (ACOTs) catalyze the hydrolysis of CoA esters leading to the production of free fatty acids and CoA. It is hypothesized that ACOTs are critical in regulation of intracellular levels of CoA and fatty acids. Regulation of ACOTs by circadian clock mechanisms is not well studied. In particular, the mechanism by which circadian clock proteins cryptochrome (CRY) are involved in ACOT protein expression is understudied. To study the effect of feeding regimen on ACOT expression we sampled tissue from wild-type (WT), and CRY 1,2 double knockout mice. Both genotypes were also tested based on different feeding regimen; either *ad libitum* (AL) or 30% calorie restricted (CR). Effects on aging and the circadian clock from CR feeding regiments is well studied and thus critical to test when investigating metabolic pathways controlled by circadian clock proteins.

INTRODUCTION

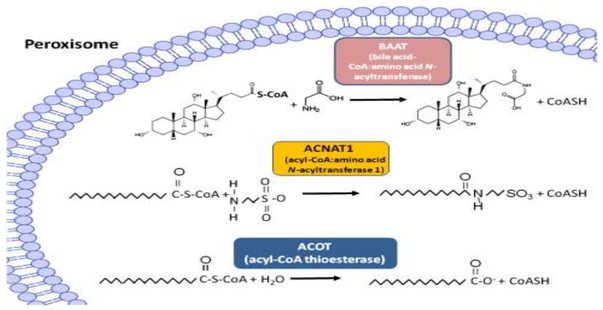

Fig 1. ACOT proteins catalyze the oxidation of CoA esters into short-chain fatty acids and CoASH in the peroxisome. Credit: *The emerging role of acyl-CoA thioesterases and acyltransferases in regulating peroxisomal lipid metabolism*. Sept. 2012. Web. *Science Direct*.

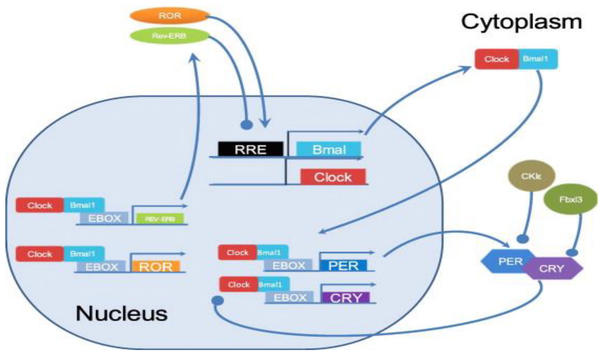

Fig 2. The intrinsic circadian clock regulatory pathway involves the regulation of several genes. Cryptochromes (CRYs) are critical in the regulation of circadian rhythms. It was hypothesized that ACOT protein expression may be effected by CRY. Credit: *Molecular Mechanisms of the Circadian Clockwork in Mammals*. Aug. 2014. Web. *Science Direct*.

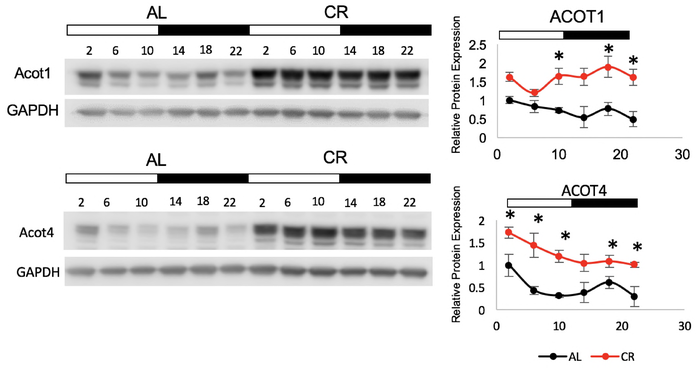

Fig 3. Previous Western blot analysis from our lab indicates significant upregulation in the expression of ACOT proteins based on different feeding regimens. However, the regulation by CRYs was not yet explored. Graphs represent relative expression values; an asterisk indicates statistical significance.

EXPERIMENTAL DESIGN

TWO FEEDING REGIMENS:

1. **Ad-Libitum Control** – mice have unlimited access to food; able to feed at any time in the day
2. **30% Calorie Restriction** – Food provided at ZT14 every day. CR feeding regimen provides enough food to prevent malnourishment.

METHODS

- Breed mice with both wild-type (WT) and *Cry1,2*$^{-/-}$ genotypes
- Place all mice on either *Ad Libitum* (AL) or calorie restricted (CR) feeding regimens
- At maturity, mice were euthanized at all of the indicated time points allowing for protein and mRNA expression analysis across the day
- Tissue collection was performed
- Protein and RNA isolation procedures were followed
- RNA was reverse transcribed to cDNA to quantify *ACOT* gene expression by using real-time PCR
- Protein was subjected to western blot analysis to visualize and quantify ACOT protein expression

RESULTS

Cry1,2$^{-/-}$ Mice Show Increased Expression of ACOTs

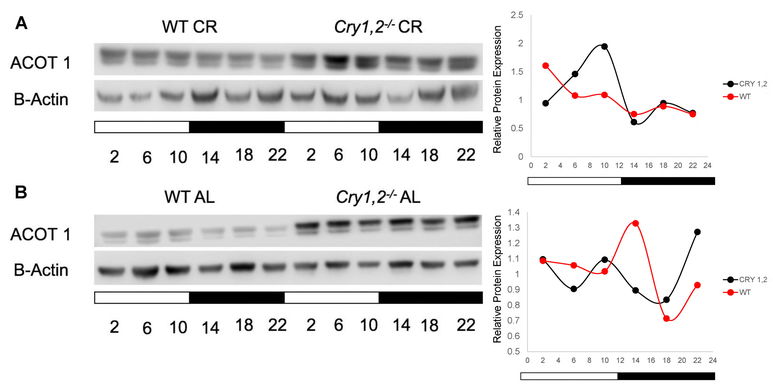

Fig. 4 A, & B: ACOT1 protein expression is upregulated in *Cry1,2*$^{-/-}$ mice. Western blot and quantitative analysis for total ACOT1 protein was performed on the liver tissue obtained from mice subjected to Ad libitum feeding (AL) and 30% calorie restricted (CR). All time points were normalized against B-actin control to ensure accuracy.

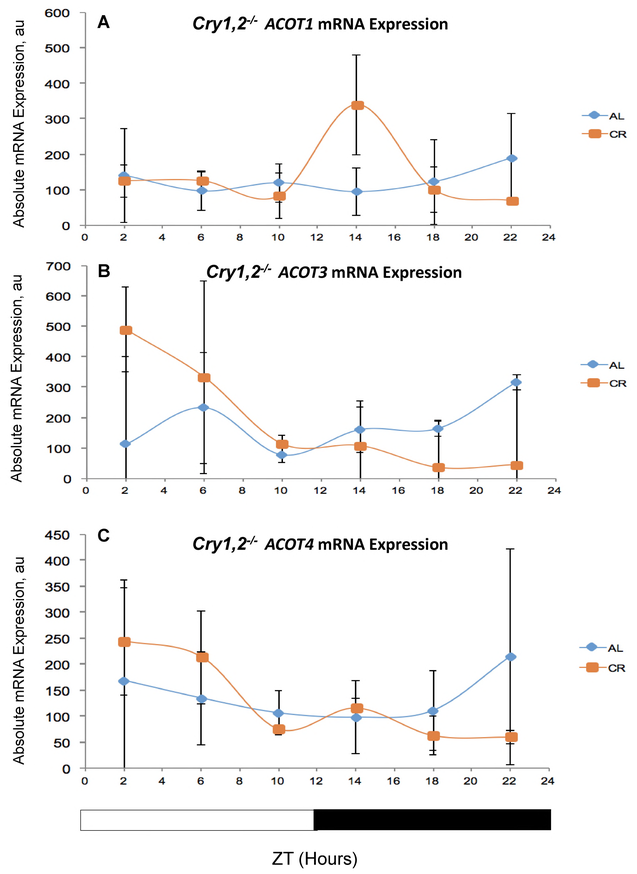

Fig. 5 A, B, & C: mRNA expression is not different between feeding regiments. The graphs represent the expression of ACOT1 protein under different feeding regimens. Total mRNA was extracted from liver tissues of mice on Ad libitum feeding (AL) and calorie restricted (CR) feeding. mRNA was reverse transcribed to cDNA and amplified for gene expression by Real time PCR. 18s ribosomal RNA was used as internal control. Each time point represents 3 mice. Data for AL and CR are plotted for comparison to one another to determine effect from feeding regimen.

SUMMARY

- *Cry1,2*$^{--}$ knockout mice have lower body weight
- Expression of *ACOT1* mRNA is similar in *Cry1,2*$^{-/-}$ mice regardless of feeding regimen
- ACOT1 protein expression levels are similar across the day but are significantly upregulated at specific time points in double knockout mice
- Wild type (WT) mice have reduced expression of ACOT proteins when compared to *Cry1,2*$^{-/-}$ mice

FUTURE DIRECTIONS

- Perform quantitative western blot analysis in order to verify upregulation of ACOTs through statistical analysis
- Perform PCR analysis on WT mRNA to compare expression levels to double knockout mice
- Investigate the effect of feeding regimen and clock interactions in knockout mice
- Investigate the biochemical pathway by which circadian clock genes influence ACOT expression
- Analyze PCR results for error and perform multiple times to verify results
- Verify *CRY1,2*$^{-/+}$ do not also exhibit ACOT upregulation and that double knockout genotype is responsible